Original Paper

Study on the Special Components of Rice Bran Oil (I)

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Synopsis: Unsaponifiable matters of rice bran oil, especially the components of oryzanols were researched. The fractionation of triterpenols and concentrating of them in the edible rice bran oil were studied, and the most bioactive component, cycloartenol ferulic acid ester (CFE) was identified. The methods of concentrating CFE in edible rice bran oil were discussed. Bioassay of CFE was tried.

1. Introduction

The rice bran oil report was first published by A. Smetham (1898)[1]. Also T. Hoshi (1908)[2] and M. Tsujimoto (1908)[3] in Japan reported about industrial sample. S. Ueno and T. Tsuchiya et al.[4)~74)] published many papers. The industry of rice bran oil developed in Japan before the Second World War, and annual production of rice bran crude oil amounted twenty thousands metric tons as the top of the world, however recent global production reached to more than a half million tons.

The fatty acid composision of Japanese rice bran oil is generally as follows: 45% oleic acid, nearly 1% gadoleic acid, 35% linoleic acid and about 1% n-6 linolenic acid as unsaturated or polyunsaturated fatty acid. On the other hand, as the saturated acids, 17% palmitic acid, 1+% stearic acid, and less than 1% arachidic acid are components, by Y. Takeshita's statistical analysis. However, about overseas oils some differences[6)7)], are appeared. These composisions show the glycerides are semidrying oleic oil, and are expected as suitable for edible purpose.

Another and most useful character is unsaponifiable matter components. Wax, fatty acid ester of monohydric alcohol or hydrocarbon, tocopherol, tocotrienol and oryzanol or ferulic ester of triterpene alcohol, sterol or phospholipid are all refered to USM of rice bran oil.

Oryzanol is new and most physiological substance discovered, researched and developed at Japanese National Chemical Research Laboratory by T. Tsuchiya, R. Kaneko, A. Kato, A. Tanaka, T. Mamuro, K. Tanabe, O. Okubo, T. Endo and many others.[8)] The authors, Y. Takeshita and H. Naruse contributed to the research of related substance of oryzanol, and industrializing of oryzanol and CFE or its effective component concentrating, as the assistant of the late T. Tsuchiya or his coworker until recent decade. S. Suzuki[9)] discovered the influence of rice bran edible oil to serum cholesterol by clinical test, and confirmed that this effect was caused to the unsaponifiable matter in oil.

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In USA, at Southern Regional Research Lab. and Western Regional Research Center of USDA, between early decade after Second World War and recent years, several papers were published. Especially the late R. M. Saunders and R. N. Sayre[10] or R. J. Nicolosi et al. of Lowel Univ.[11] researched the stabilizing by extruding raw rice bran for developing crude oil or decrease of serum cholesterol, mainly by bioassays, and discussed also effective components of oryzanol, for example cycloartenol ferulic ester.

2. Experimental

2.1 Oryzanol Concentrating in Edible Rice Bran Oil

Ordinary vegetable oils were able to minimize acid vaue less than 0.03 through deodorization process, however about rice bran oil man could not be reduce AV from ca. 1.0 AV of bleached step or after physically deacidified oil to 0.03. Only alkali refining can reduce AV to this range. Y. Takeshita[12] solved this problem by the appeal of high boiling point acidic component, that is ferulic ester, oryzanol. He discovered double acid values of rice bran oil, and lower AV concerned to fatty acid which was measured with alkaliblue-6B or bromothymolblue indicator and higher value which was given by phenolphthalein or thymolphthalein indicator owed to fatty acid plus phenolic OH of ferulic ester represented by oryzanol.

Generally the AV of edible oil is oilchemically defined as titrated value of KOH mg with phenolphthalein indicator about 1.00 gram oil sample, but by Takeshita the true AV of rice bran oil concerning to free fatty acid may be the AV by alkaliblue-6B, and the difference to phenolphthalein AV refers to mainly to ferulic acid component. By this theory, chemical process for enrich of orizanol or fractionated triterpenol ester for example cycloartenol ferulic ester was developed.

The enrichment of oryzanol are valuable dietetically and pharmaceutically, and above mentioned method by the author et al. was registered as the US patent.[13]

Another chemical method and physical process are patent pending. Physical process is

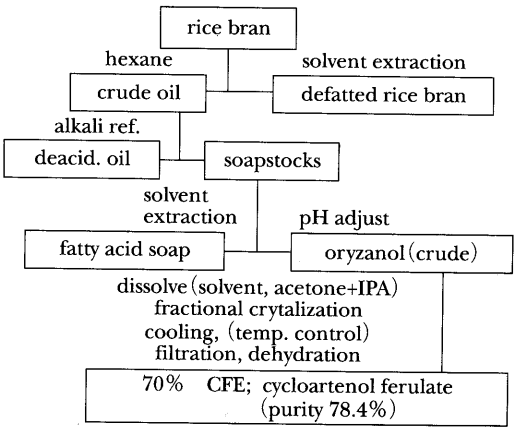

Fig. 1 Process for Oryzanol and CFE

more simple than chemical process, however apprehension of thermal denaturation of oryzanol is a demerit of it, and also bleaching cost is not low, inspite of high yield of deacidified oil, and low cost of waste disposal.

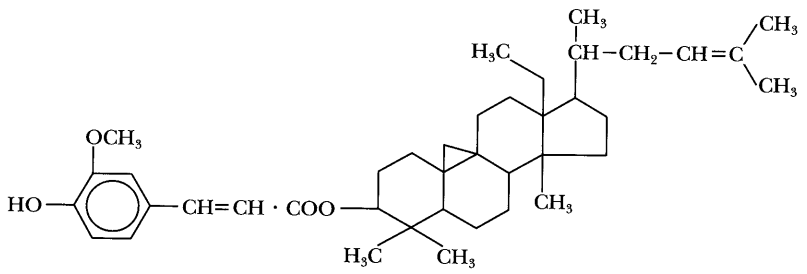

Fig. 2 Chem. structural Formula of CFE

2.2 Fractionation of Oryzanol by HPLC

Earlier identification of oryzanol in National Chemical Laboratory was completed by initially column chromatography, thin layer chromatography and finally checking by UV absorption spectra, and the difinition of oryzanol was physiological active substance which showed absorption maxima at near 231, 291 and 315 nm. However, those UV absorption belong to conjugate double bonds of ferulic acid, and oryzanol is the ferulic ester of triterpene alcohol. By these analytical results, GLC fractionation of sterol and triterpenol were tested, but the most useful analysis without thermal denaturation was developed by A. Tanaka et al.[14] and K. Tanabe et al.[15] by HPLC at near ambient temperature.

The author H. Naruse[16] developed the isolation of physiological active ferulate of triterpenols by HPLC analytical method at 40℃ and confirmed medically cycloartenol ferulate as most effective component of γ-oryzanol.

The author H. Naruse developed not only CFE fractionation from γ-oryzanol but confirmed neutritional and pharmaceutical effect of CFE comparing to ordinary γ-oryzanol with physiological activity by the assistance of medical coworker.

2.3 Fractionation and Refining of CFE

The composition of γ-oryzanol as the raw material of CFE adopted by the author Naruse contented CFE 47.2% and 24-methylene artanol 51.8%.

2.3.1 Acetone/IPA Method

After dissolving 100 g γ-oryzanol with acetone/IPA (1 : 1 v/v) 500 mL at 60℃, solution was colled to 35℃, and CFE crystal deposited 30.6 g, primary. (purity CFE 79.4%, 24-Me 19.2%) After recrystallization, 15.2 g CFE was obtained, and its composition was pure CFE 97.5% and 24-Me 2.0%. In these experimens, 10, 15 or 20% w/v γ-oryzanol solution was compared, however recognized no difference, and as the result 20% solution was choised finally.

Comparing to wet solvent, dry solvent gave better effect. Rapid cooling of γ-oryzanol solution gave much impurities in deposited CFE crystal.

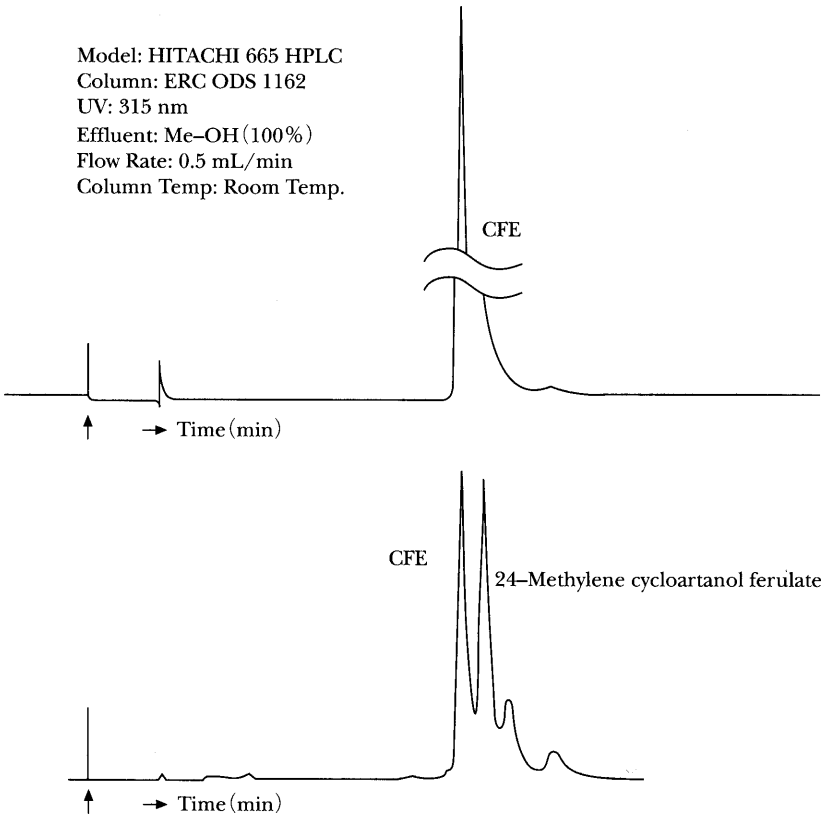

Fig. 3 HPLC of γ-Oryzanol and CFE

2.3.2 Acetone Methanol Method

γ-oryzanol 100 g was dissolved in acetone 600 mL at 60℃ and after adding 1% methanol, solution was cooled in a refrigerator. Crystal deposited as follows.

Table 1 Result of Acetone Recrystalization

Treating No. of Times	Deposited Weight(g)	CFE Crystal Purity(%)
1	84.3	67.7
5	50.0	82.1
10	32.6	92.2

2.3.3 M. E. K. Method

γ-oryzanol 100 g dissolved in M. E. K. 800 mL at 70℃. And then water or methanol was added and cooled in refrigerator.

Deposited crystal was as follows.

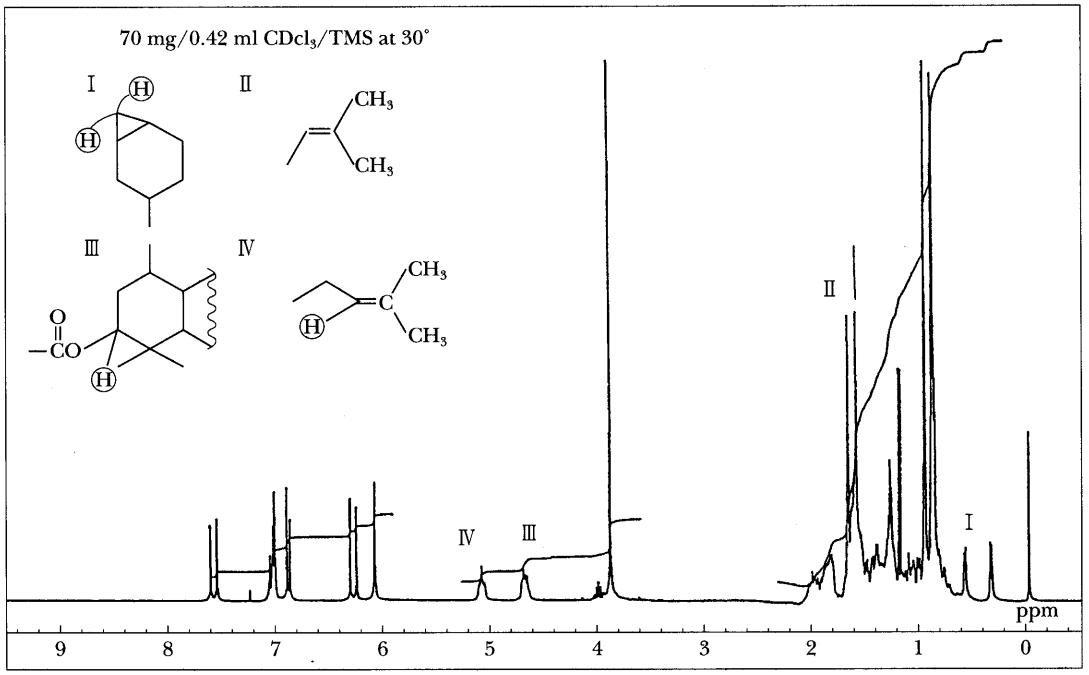

Fig. 4 NMR spectrum of CFE
(Model: HITACHI R-1500)

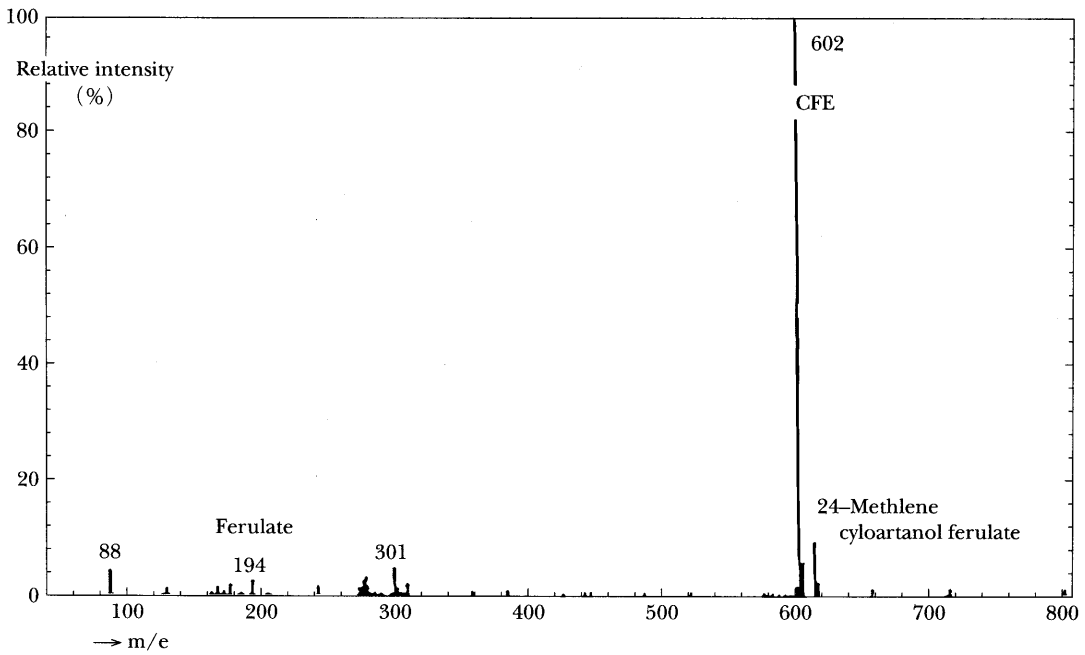

Fig. 5 Mass Spectrum of CFE
(Model: Shimazu QP-5000)

Table 2 Recrystalization of CFE

Solvent	Composition		Deposited Crystal wt(g)	Purity(%)
M.E.K.(800 mL)	mL	%	12.0	68.4
M.E.K.+water	8	(1)	39.2	59.9
+water	40	(5)	57.6	53.2
M.E.K.+methanol	8	(1)	29.6	60.0
+methanol	40	(5)	37.6	59.2

2.4 Toxicity Test for Fractionated CFE

Concentrated CFE was used for medical test as follows.

1), Acute Toxicity Test; LD_{50} Rat, oral 10,000 mg/kg

2), Subacute Toxicity Test; 30 days negative (oral 2,890 mg/kg)

3), Chronic Toxicity Test; max. 1,000 mg/kg/day, oral negative.

4), Mutagenesity Test; negative.

5), Freak of Nature; negative.

2.5 CFE Content and Medical Effect

2.5.1 Method of Experiment

Using 4 week age SD rat, serum lipid controling test was carried out, about the groups of cholesterol feed (control), CFE contents 40, 50, 60, 70, 80, or 97%. About CFE containing group, metallic sampling device was served and continued between 12 days. Sample dose was each 1,000 mg/kg.

2.5.2 Results of Tests

It was confirmed that effect of CFE to arterioselerosis index was significant and reasonable at more than 70% cont. as following Table 3.

Table 3 CFE to Arterioselerosis Index

CFE cont.	Arterioselerosis Index*	Significant Reasonable Error
control	15.2	
40%	14.6	
50	14.8	
60	14.2	
70	8.0	$p<0.05$
80	7.8	$p<0.01$
97	7.2	$p<0.001$

* Arterioselerosis Index $=\frac{(TC)-(HDL\text{-}C)}{(HDL\text{-}C)}$

Anticholesterol reaction of CFE: Several kinds of drug are available for this intention, but as those have often ill reaction of lever impediment, there is a problem of long period dose. On the other hand γ-oryzanol was reported as some serum lipid decreasing by phar-

macological prove. (Geriat)[73].

About anticholesterol reaction of γ-oryzanol Yamamoto[74] infered lipid excretion promotion to bile. This is the action as cholesterol inhibitor. However, this has some question, as the report about above mentioned reference of solubility and absorption to efficiency. In this report serum lipid level lowering was detected, and then CFE was evident to have more superior effectiveness than γ-oryzanol.

3. Results and Discussion

Rice bran edible oil is only one vegetable oleic oil which contains much unsaponiable refering to ferulic ester of triterpenol, that is oryzanol. Authors studied to concentrate or isolate physiologocal active components, and method to concentrate most active triterpenol ferulate so called cycloartenol ferulate in edible refined oil.

γ-oryzanol, registered pharmaceutically is a mixture of ferulate, but authors appointed as most active ferulate, CFE and could concentrate in refined rice bran oil.

4. Conclusion

The method of isolation and concentration in edible refined rice bran oil were invented and developed by chemical process, HPLC etc. without thermal denaturation.

The bioassay of unsaponifiable matters was tried by colaborating of several medical researchers, and CFA was confirmed as physiological effective component.

Acknowledgement

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論　文

米ぬか油の特殊成分の研究

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要旨：米ぬか油の特殊成分である不けん化物，特にオリザノールの研究をした。その成分，トリテルペンアルコール類の分画により生理的に最も作用の強いシクロアルテノールフエルラ酸エステル (CFE) の分離と油中での濃縮に就いて成果をえた。更に動物試験に依る効果の確認を試みて成果を得た。

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